Takashi MATSUSHIMA: **Production of chlamydospore-like bodies by a solopathogenic line of Ustilago maydis Corda***

松島 崇: *Ustilago maydis* の単独病源系による厚膜胞子様体の形成について*

Ustilago maydis (DC.) Corda, the common corn smut fungus, is normally heterothallic, and its galls and chlamydospores (zygotes) are produced when corn is inoculated with a pair of compatible haploid lines. Solopathogenic lines were described by Christensen (1) and Eddins (2) as monosporidial lines which produced by themselves galls and chlamydospores in inoculated corn. The cultural, sexual and pathogenic segregants obtained during germination of the chlamydospores produced by these lines are identical to those recovered from chlamydospores of a cross of two haploid lines (3, 6). Since cells of solopathogens are uninuleate (1), they are considered to be diploid, although cytological proof of the presence of $2n$ chromosomes has not been obtained.

Very detailed studies (3) have established that sexual compatibility in *Ustilago maydis* is governed by genes at two loci, one of which has only two known alleles (a^1, a^2), while the other has multiple alleles (b^1, b^2, b^3,……b^n). Mated haploid lines produce galls and chlamydospores in corn only if different a and b are brought together. The sex genotype of solopathogenic lines is represented as $a^1\ a^2\ b^\alpha\ b^\beta$.

The chlamydospores of *Ustilago maydis* are produced through three distinct stages, i. e., plasmogamy (sporidial fusion or hyphal fusion) of two sexually compatible haploid lines, development of the resultant dikaryotic phase, and karyogamy. In *Ustilago maydis*, the part of the life cycle from plasmogamy to karyogamy is parasitic phases in corn plants. Although the plasmogamy itself is possible to induce in artificial cultures (4, 5), but the resultant dikaryotic phase is quite unstable in artificial cultures and within 24 hours it will separate into the component haploid lines (5). But in the solopathogenic lines, the induction of chlamydospores in artificial cultures might be possible because of the probable diploid of the vegetative cells.

To induce the sporulation of fungi, it is a general practice to put the fungi in unfavorable environmental conditions for growth. After extensive investigations according to this line, the induction of a chlamydospore-like structure in a solopathogenic

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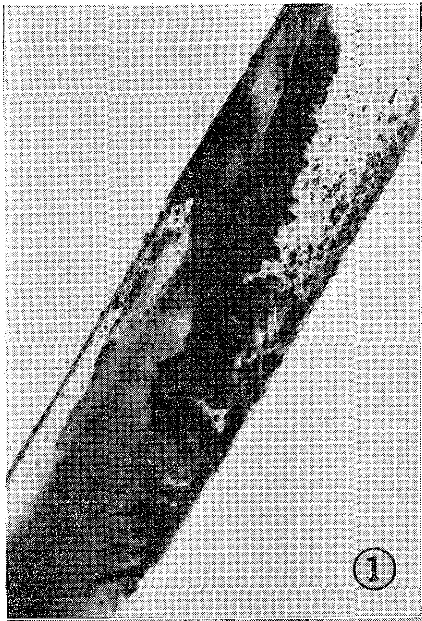

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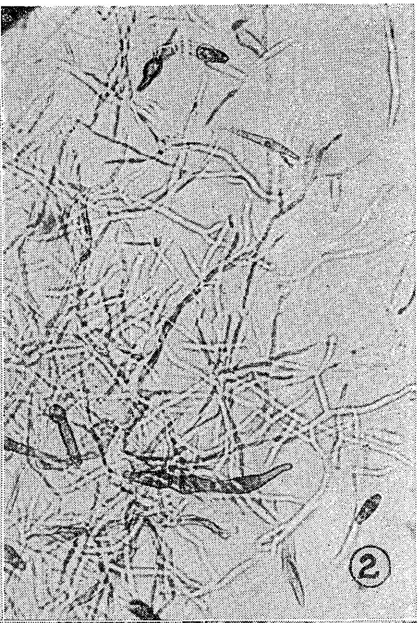

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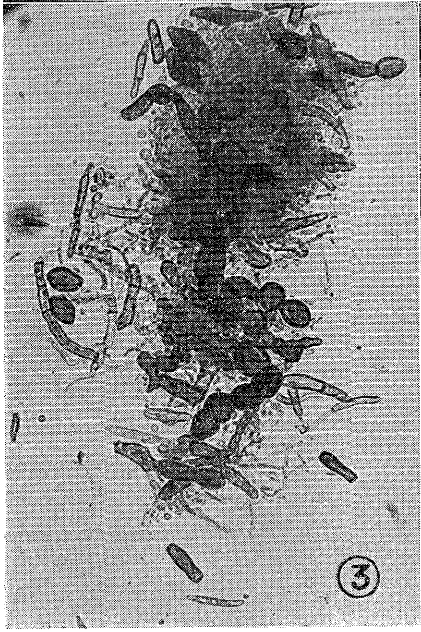

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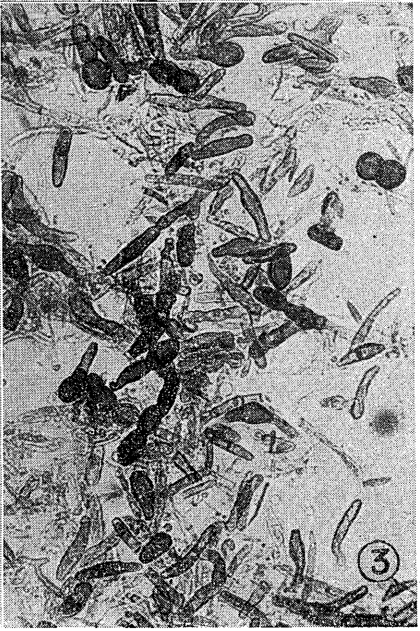

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line was achieved by adding cupper sulfate to a liquid shake culture medium.

The monosporidial lines of *Ustilago maydis* used in this study were listed below.

Haploid line	Sex-genotype	Haploid line	Sex-genotype
10A4	$a^1 b^1$	17D4	$a^2 b^4$
JvBa	$a^1 b^2$	ArBa	$a^2 b^{14}$
JoEb	$a^1 b^5$	JpDa	$a^2 b^{19}$
KrB_3	$a^1 b^7$	Solopathogenic line	
ApAa	$a^2 b^3$	410n	$a^1 a^2 b^1 b^4$

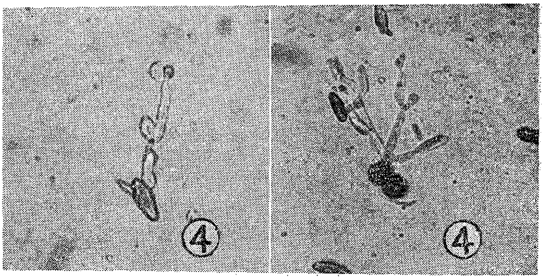

Fig. 4. Germination of the chlamydospore-like bodies.

The solopathogenic line 410*n* was isolated from a cross of lines 10A4×17D4, in 1943 by Stakman et al. (unpublished data of the Department of Plant Pathology, University of Minnesota), and the character of solopathogenicity has remained unchanged in stock cultures since that time.

All these lines were shake cultured in 20 mm test tubes containing 10 ml. of the glutamic acid-glucose medium with various concentrations of cupper sulfate at 25°C. The shake machine used in this experiment was a reciprocating type with 65 mm amplitude and 90 frequencies per minute, and test tubes were set keeping 22.5 degrees to the horizontal plane. The composition of the medium was:

Glutamic acid	5.0 gm.	Mn (from $MnSO_4$)	0.10 mgm.
Glucose	25.0 gm.	Cu (from $CuSO_4$)	0.05 mgm.
K_2HPO_4	1.0 gm.	Mo (from ammonium molybdate)	0.05 mgm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.	Agar	15 gm.
Fe (from $FeCl_3$)	0.20 mgm.	Distilled and deionized water, to make.	1000 ml.
Zn (from $ZnSO_4$)	0.20 mgm.	pH adjusted to 6.0 before autoclaving.	

The concentrations of $CuSO_4 \cdot 5H_2O$ were 0, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 and 1000 p.p.m. by adding Seitz-filtered $CuSO_4 \cdot 5H_2O$ solutions of

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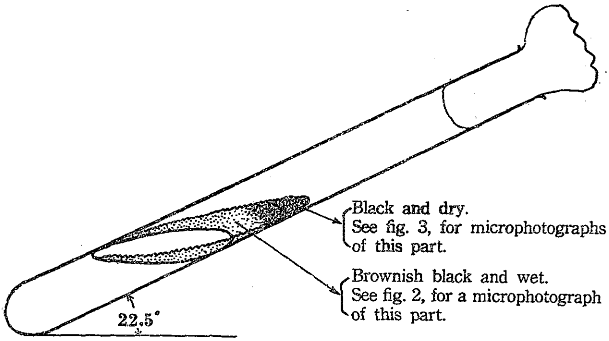
Fig. 1. Formation of the black fungal ring on a test tube wall (enlarged).

Figs. 2 and 3. Chlamydospore-like bodies ×370.

appropriate concentrations to the autoclaved medium.

After about 10 days' shake culture at 25°C., 410n cultures with added $CuSO_4 \cdot 5H_2O$ of 150 to 350 p.p.m. produced black fungal rings on test tube walls with best formation at 150 p.p.m. (Fig. 1 and Fig. 5). No haploid lines tested produced this structure.



Black and dry. See fig. 3, for microphotographs of this part.

Brownish black and wet. See fig. 2, for a microphotograph of this part.

Fig. 5. The illustration of a black fungal ring.

The microscopic examination of this black fungal ring was shown on Fig. 2 and Fig. 3, referring the positions of the fungal ring to Fig. 5. This chlamydospore-like bodies were closely resembling those produced in inoculated corn plants in size, mode of formation and germination (Fig. 4), except the echinuation of the spores.

With these facts, this may be the first success of producing the chlamydospore of *Ustilago maydis* in artificial culture. In order to confirm this, further investigations are carrying on at present time.

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